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Research Paper

Results of Use of Tissue-Engineered Autologous Oral Mucosa Graft for Urethral Reconstruction: A Multicenter, Prospective, Observational Trial

Gouya Ram-Liebig [a,*], Guido Barbagli [b], Axel Heidenreich [c], Dirk Fahlenkamp [d], Giuseppe Romano [e], Udo Rebmann [f], Diana Standhaft [f], Hermann van Ahlen [g], Samer Schakaki [g], Ulf Balsmeyer [d], Maria Spiegeler [h], Helmut Knispel [h]

[a] UroTiss Europe GmbH, Otto-Hahn-Str.15, 44227 Dortmund, Germany
[b] Centro Chirurgico Toscana, Via dei Lecci, 22, 52100 Arezzo, Italy
[c] University Clinic and Policlinic for Urology, Kerpener Str. 62, 50937 Cologne, Germany
[d] Zeisigwald Clinics Bethanien, Department of Urology, Zeisigwaldstrasse 101, 09130 Chemnitz, Germany
[e] Urology Unit, Ospedale del Valdarno, Santa Maria alla Gruccia, Piazza del Volontariato, 1, 52025 Montevarchi-Arezzo, Italy
[f] Diakonissen Clinics Dessau, Department of Urology, Gropiusallee 3, 06846 Dessau-Roßlau, Germany
[g] Osnabrueck Clinic, Department of Urology, Am Finkenhügel 1, 49076 Osnabrück, Germany
[h] St. Hedwig Hospital, Department of Urology, Große Hamburger Strasse 5-11, 10115 Berlin, Germany

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ABSTRACT

Background: Harvest of oral mucosa for urethroplasty due to urethral stricture is associated with donor-site-morbidity. We assessed functionality and safety of an authorized tissue-engineered oral mucosa graft (TEOMG) under routine practice in stricture recurrences of any etiology, location, length and severity (real-world data).
Methods: 99 patients from eight centers with heterogenous urethroplasty experience levels were included in this prospective, non-interventional observational study. Primary and secondary outcomes were success rate (SR) and safety at 12 and 24 months.
Findings: All but one patient had ≥ 1, 77.1% (64 of 83) ≥ 2 and 31.3% (26 of 83) ≥ 4 previous surgical treatments. Pre- and postoperative mean ± SD peak flow rate (Qmax) were 8.3 ± 4.7 mL/s (n = 57) and 25.4 ± 14.7 mL/s (n = 51). SR was 67.3% (95% CI 57.6–77.0) at 12 and 58.2% (95% CI 47.7–68.7) at 24 months (conservative Kaplan Meier assessment). SR ranged between 85.7% and 0% in case of high and low surgical experience. Simple proportions of 12-month and 24-month SR for evaluable patients in all centers were 70.8% (46 of 65) and 76.9% (30 of 39). Except for one patient, no oral adverse event was reported.
Interpretations: TEOMG is safe and efficient in urethroplasty.

1. Introduction

Urethral stricture affects up to 0.6% of the male population with significant disease burden (Alwaal et al., 2014; Liu et al., 2016; Wessells et al., 2017; Latini et al., 2014). Except for the guidelines of the American Urological Association, no therapeutic recommendations exist (Wessells et al., 2017; Latini et al., 2014). These guidelines are mainly based on expert opinions and publications of lower evidence strength grades due to the lack of data obtained from prospective multicenter trials under good clinical practice (GCP) standard (Latini et al., 2014; Mundy, 2006; Tritschler et al., 2013). Consequently, different surgical techniques are applied according to the surgeon's preference and previous experience. Over the last two decades, buccal mucosa became the tissue of choice for urethral reconstruction (Wessells et al., 2017;

Ram-Liebig et al., 2015; Markiewicz et al., 2007). However, oral mucosa harvest may lead to donor-site morbidity (Ram-Liebig et al., 2015; Jang et al., 2005; Fasolis et al., 2014; Markiewicz et al., 2008).

Tissue-engineered oral mucosa graft (TEOMG) represents an alternative material for urethroplasty. It helps to avoid morbidities associated with graft harvesting at the oral site and provides substitution tissue for urethral reconstruction in any size required (Ram-Liebig et al., 2015). We conducted an observational study with a TEOMG, with market authorization in Germany (MukoCell®), to expand the knowledge about feasibility, safety, and efficacy when used under routine real-world conditions in non-preselected adult male patients with surgically unsuccessful pretreated urethral stricture. The current data from our observational trial are reported to the Paul-Ehrlich-Institut, the regulatory body in Germany, responsible for marketing authorization of advanced therapy medicinal products (ATMP) - among others - and approval of clinical trials, as well as to the European Medicine Agency (the European Union agency for the evaluation of medicinal products).

* Corresponding author.
  E-mail address: g.ram-liebig@urotiss.com (G. Ram-Liebig).

2. Materials and Methods

2.1. Study Design and Patients

The study is a prospective, observational survey conducted at eight German urologic centers, with <10 to >80 urethroplasties/year. This study is registered in Germany at the Paul-Ehrlich-Institut observational trial registry, NIS number 110.

Enrolled were adult male patients with recurrent urethral stricture. Decision for treatment of an individual patient with the autologous TEOMG was met solely by the treating surgeon.

All data captured during the observation were obtained from routine clinical care assessments which were done by the investigators according to their local medical practice (Real-world data), without additional, study-mandated examinations or clinic visits. The study was monitored by an independent licensed German Contract Research Organization.

The trial was designed in accordance with the Declaration of Helsinki with all its amendments. The study was approved by the local ethics committees and the competent national supervisory authority (Paul-Ehrlich-Institut, Langen, Germany). The trial followed GCP guidelines, European guidelines on ATMPs, and German Transplant Act. The patients signed informed consents for biopsy and blood taking, as well as for urethroplasty with TEOMG. The TEOMG implanted in the context of this study (MukoCell®) was provided by UroTiss GmbH, Germany.

2.2. Coordination and Schedules

For the manufacture of MukoCell®, a tiny oral biopsy is required. For being authorized to take biopsies, the urologist needs an approval according to German Drug Law from the authority, who granted the Good Manufacturing Practice (GMP) license for the TEOMG. For this, the clinic has first to provide documents to show that it has an appropriate facility. A hygiene plan, complying with the medical standards and suitable for carrying out biopsy procedures and blood collection is also required. The urologist who will have the primary responsibility for biopsy taking and blood collection, as well as the medical staff, who will be involved in the procedures must be trained for biopsy taking and blood collection and their storage as well as documentation of the procedure according to standard operating procedures, in compliance with good professional practice. Once the tissue collection authorization is available, and the patient agrees for the urethroplasty with MukoCell®, the urologist contacts the company by phone or email and informs it about the date, planned for biopsy taking and urethroplasty surgery. Within a few days, or if necessary within hours, he gets a biopsy kit from the Good Manufacturing Practice (GMP) laboratory. The biopsy kits are stable for 6 months. Together with the biopsy kit, the patient gets a unique identification code. This code is the first step of patient recruitment into the study. For tissue collection, a donor record, containing documentation of donor suitability and a patient consent form, should be completed. Once the biopsy is taken, it is put into the specific package, which is picked up at the same day. On the day after, upon arrival in the GMP laboratory, the manufacture begins. For safety reasons, the serologic examination must be negative for specific infectious agents (Human Immunodeficiency Virus, Hepatitis B and C, Treponema Pallidum), to allow release of the tissue for manufacture. Once manufacture begins, the date for urethroplasty is already fixed. According to this date, MukoCell® is placed in a sterile double package, and sent within a qualified transport container to the hospital. It must be used within 48 h.

TEOMG is an industrial product. The manufacturing processes therefore cannot be disclosed in all details. All procedures (identification code, biopsy taking, manufacturing, shipment) are standardized, validated and certified, respectively.

3. Procedures

For manufacture of TEOMG, a tiny oral mucosa biopsy of 0.5 cm^2 (Fig. 1A) was taken from patient's buccal mucosa and sent to the GMP laboratory for aseptic manufacturing of the graft, which has been described elsewhere (Ram-Liebig et al., 2015). In the manufacture site, all culture flasks, materials and documents were identified for each patient with the unique identification code, which was the same, as on the biopsy kit. All manufacture steps took place in an isolator and the culture steps in an incubator (37 °C, 5% CO_2) in the GMP laboratory. After separation from submucosa, the mucosa tissue was used for setting the cell cultures in flasks and their incubation. The expansion of cells took about two weeks. Once the epithelial cells were confluent, primary cultures were detached form the flasks and the non-splitted cells of passage 1 were seeded on a biodegradable membrane. Subsequently, the final TEOMG, consisting of oral epithelial cells from first passage cultured on biodegradable protein containing scaffold, was placed in a sterile container, packaged and pharmaceutically released for therapeutic use, after a final check of properness of quality control results and completeness of documentation. The manufacture of each batch was documented in an according protocol. All remaining materials and wastes were disposed according to specific Standard Operating Procedures. Timing of the whole procedure (3 weeks) was highly reliable, allowing to settle the surgery date as soon as the biopsy is taken from the patient. After release, the TEOMG was sent to the hospital for implantation into the patient's diseased urethra (Ram-Liebig et al., 2015). The shipment of TEOMG is a validated process, ensuring stability and viability of the tissue for 48 h.

Before urethroplasty, information on demographic and medical history was gathered. Pre- and post-operatively, results from physical examination, vital signs measurements, electrocardiogram, serological examinations, concomitant medication, and conventional urological examinations (e.g. urethroscopy, urethrography Fig. 1E, or uroflowmetry) were collected.

The TEOMG was implanted in accordance with the substitution urethroplasty technique routinely applied by the surgeon (Fig. 1B–D) when native buccal mucosa was used. After the operation, an 18 to 20 Ch. Foley silicon catheter was left in the urethra. Suprapubic catheter was placed in the urinary bladder in some cases about 3–6 weeks later, the catheters were removed and the patient underwent voiding urethrography (Fig. 1F).

Routine urological examinations such as uroflowmetry, urethroscopy and/or urethrography were usually repeated every 3 months during the first year and every 6 months during the second year unless there were symptoms of urethral re-stricture (e.g. decreased urinary flow).

4. Outcomes

The primary outcome was the success rate (SR), defined as the absence of stricture recurrence, at 12 months after TEOMG implantation. The prospectively selected definition of stricture recurrence was: evidence of a postoperative peak flow rate (Qmax) < 15 mL/s on uroflowmetry plus the urethra is not passable with a catheter (diameter = 16–18 Ch) or during standard urethroscopy. However, these diagnostic criteria did not correspond to the actual routine diagnostic practice at the participating sites, precluding the use of this definition for stricture recurrence. Therefore, a consolidated assessment of stricture recurrence was made post hoc, based on investigator rating, patient-reported spontaneous micturition after urethroplasty, and uroflow rate following urethroplasty (i.e. Qmax < 15 mL/s). The physician's assessment "treatment successful = yes" was used to exclude stricture recurrence, except in cases where patients reported difficulty of spontaneous micturition, where later re-stricture was detected, where there was need for further instrumental intervention, or where the physician's statement
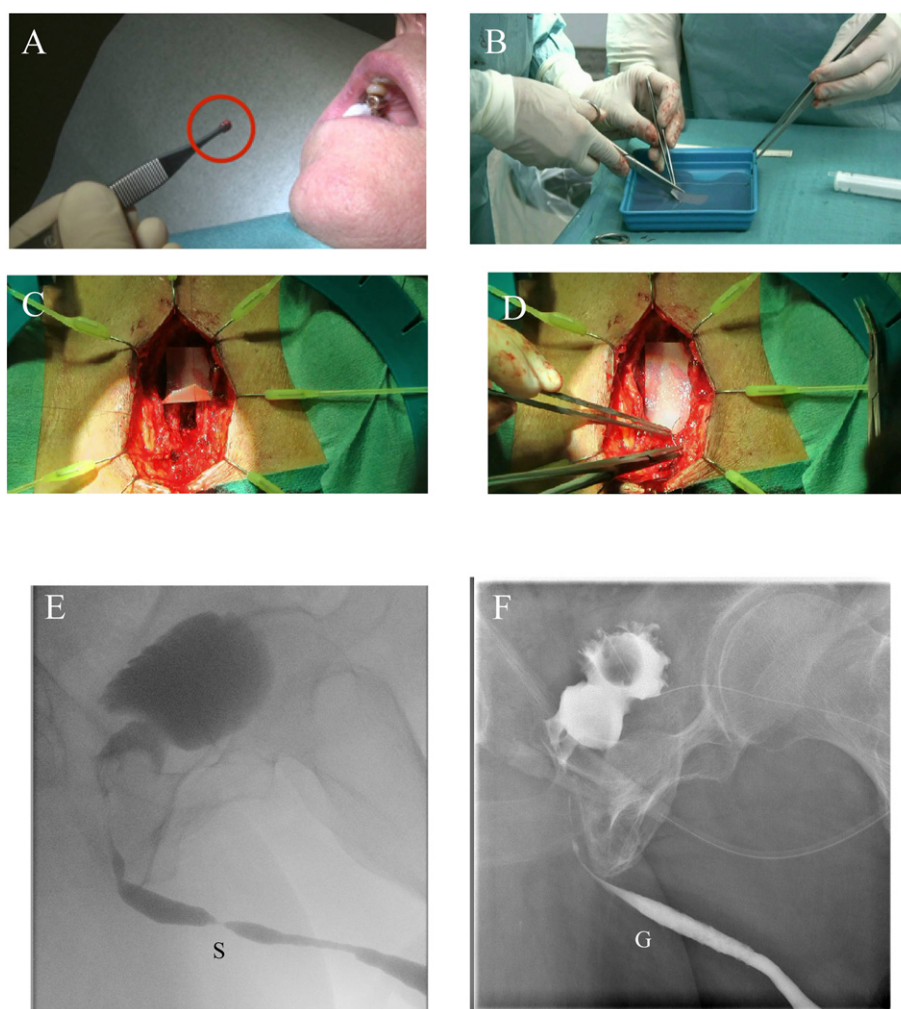
Fig. 1. Urethroplasty with the autologous tissue-engineered oral mucosa graft MukoCell®. A small oral mucosa biopsy is taken from the cheek of the patient (A) which is used for the manufacture of the graft. The latter is cut into the desirable size (B), transferred to the opened urethra (C) and sutured as a ventral onlay graft (D). Pre- (E) and postoperative (F) voiding urethrography, before and 3 weeks after the implantation of autologous tissue-engineered oral mucosa graft. The strictured (S) and grafted (G) area are indicated in (E) and (F).

was missing in the case report form. In the latter situation, any evidence of re-stricture detected during monitoring was used to assess treatment failure, unless re-stricture was clearly outside the operated urethral area (i.e. heterotopic recurrence). In the absence of such evidence in these patients, the outcome was considered successful (i.e. no stricture recurrence). As a sort of sensitivity analysis, available flow rates separately based on uncensored data were analyzed, thereby using the objective outcome component of the primary endpoint (i.e. $Q_{max} < 10$ mL/s) as measure for stricture recurrence (Mundy, 2006). For this paper, uncensored data analysis was also assessed utilizing age-related Q_{max} (Abrams et al., 1987) as evidence of stricture recurrence.

Secondary outcomes were SR at 3, 6, 18, and 24 months after TEOMG implantation, proportion of patients with spontaneous urination at 24 h after removal of the intraoperatively inserted catheter, and Q_{max} at catheter removal and 3, 6, 12, 18, and 24 months after urethroplasty. Safety endpoints included adverse events, frequency of perioperative complications (at oral and urethral sites), vital signs and electrocardiogram data. In addition, oral pain was recorded within 10 days postoperatively, and at 3, 6, 12, 18, and 24 months as assessed by the patients on a 4-point Likert scale (1 = none, 2 = mild, 3 = moderate, 4 = severe pain). All adverse events, either local or systemic, were handled

according to GCP guidelines of the International Conference on Harmonization (ICH-GCP guideline E6 [R1]).

4.1. Statistical Analysis

We calculated Kaplan-Meier estimates of stricture-free survival with corresponding 95% confidence intervals (CIs) for median event times. In the Kaplan-Meier analysis, no evidence of treatment failure at the time of last observation was classified as censored observation. In case of doubt, treatment failure was assumed. The proportion of patients (crude rate) with treatment success was also assessed, accompanied by the 95% CI, for all time points evaluated. For this uncensored analysis, missing data were imputed by using the last observation carried forward method. For patients with no evidence of treatment failure at a time of last observation occurring before the analysis time point, absence of stricture recurrence during the observation gap was confirmed by retrospectively screening hospital records, and assessed a recurrence in unclear cases. Otherwise, all data analyses were done using summary descriptive statistics. Data from all sites were pooled and analyzed based on the full analysis set (FAS; all enrolled patients who received the TEOMG [MukoCell®] and had at least one assessment post-surgery) and safety population. The FAS set was identical to the safety analysis set. A Cox

proportional hazard analysis was also conducted based on uncensored data with the covariates age (<46, 46–65, >65 years), body mass index (normal, overweight, obese), number of prior urethrotomies and/or urethroplasty surgeries (1, 2–3, ≥4 surgeries), length of stricture (<20, 21–40, >40 mm), and duration of catheterization (<21, 21–27, >27 days) to estimate the impact of these factors on outcome. A two-sided p value < 0.05 was considered significant. SPSS version 22 was used for analyses.

The sample size was driven by the precision (width) of the 95% CI for the true proportion of the SR/outcome of the urethroplasty procedure with TEOMG. Using the formula n = required sample size, z-value of normal distribution, α = alpha error, d = widths of 95% CI, and p = outcome, a sample size of 100 evaluable patients was needed for a precision d = 0.18 (18%) based on an estimated treatment success of p = 70% (12 months after urethroplasty procedure) and a two-sided α of 0.05. This corresponded to a 95% probability that the (true) population outcome rate p is within the limits of the 95% CI. The estimated treatment success was based on a published study on native (oral mucosa) urethroplasty outcome (Meneghini et al., 2001).

5. Results

Eligible men from eight German hospitals and aged between 22 and 86 years were recruited in the study and were treated with TEOMG. The number of patients by center varied between 5 and 27. Patients who already had an identification code, but were not operated because of reasons such as patient's decision changing, non-confirmation of urethral stricture diagnosis, positive serology for screened infectious agents were excluded from the study.

Table 1
Patients' baseline characteristics.

	Total population (n = 99)
Age (years)	55.9 (14.8)
≤50	37 (38.1%)
51–60	21 (21.7%)
61–70	20 (20.6%)
≥71	19 (19.6%)
Missing data	2
Body-mass index (kg/m²)	27.9 (4.2)
Missing data	4
Aetiology of stricture	
Iatrogenic	36 (42.4%)
Idiopathic	9 (10.6%)
Trauma	5 (5.9%)
Other	4 (4.7%)
Unknown	31 (36.5%)
Missing data	14
Site of stricture	
Bulbar[a]	73 (82.0%)
Penile[b]	16 (18.0%)
Missing data	10
Length of stricture (mm)	38.0 (23.4)
≤20	28 (30.8%)
21–40	35 (38.5%)
≥41	28 (30.8%)
Missing data	8
Previous surgical intervention (urethrotomy and/or urethroplasty)	
None	1 (1.2%)
1	18 (21.7%)
2–3	38 (45.8%)
≥4	26 (31.3%)
Missing data	16

Data are mean (SD) or number (%); missing data are excluded from all percentage calculations.
[a] Including 3 patients with involvement of the membranous urethra.
[b] Including patients with bulbo-penile strictures (n = 6) and patients with multiple strictures including a penile one (n = 6).

Results are available for 99 patients. In total, 65 patients (65.7%) and 39 patients (39.4%) reached 12 and 24 months of follow-up, respectively. Patients' baseline characteristics are shown in Table 1.

The etiology of strictures was most frequently iatrogenic but was unknown in approximately one-third of study participants. In most patients, the stricture was located in the bulbar urethra (bulbar: 82.0%; penile: 18.0%); stricture length ranged between 5 and 130 mm with an overall mean of 38.0 mm. All but one evaluable patient had at least one, and approximately 77% of evaluable patients had at least two previous surgical treatments (urethrotomy and/or urethroplasty) for their urethral stricture.

The overall SR at 12 months (primary outcome) varied between centers with the tendency that sites recruiting fewer patients had a higher proportion of stricture recurrence cases (Table 2). Three surgeons operated in the centers 1, 3, and 4 (2 surgeons/center). In the centers 2, 5, 6, 7 and 8 always one surgeon performed all operations.

In the majority of patients, we observed no stricture recurrence during the observation period with SR of 67.3% (95% CI 57.6–77.0) at 12 months, and 58.2% (95% CI 47.7–68.7) at 24 months (Kaplan-Meier analysis (Fig. 2A)). The success rate ranged between 85.7% in the case of high and 0% in the case of low experience in the surgical method.

The uncensored analysis provided similar SR with 69.4% (68 of 98 patients, 95% CI 59.3–78.3) at 12 months and 62.2% (61 of 98, 95% CI 51.9–71.8) at 24 months. In the uncensored sensitivity analysis, utilizing a Qmax of <10 mL/s as evidence of stricture recurrence, 12-month and 24-month SR were 72.8% (67 of 92, 95% CI 62.6–81.6) and 67.4% (62 of 92, 95% CI 56.8–76.8), respectively, indicating that results from the primary analysis are conservative in nature. In this paper, due to the high average patient age, we also assessed uncensored data analysis utilizing age-related Qmax (Abrams et al., 1987) as evidence of stricture recurrence as shown in Fig. 2B. Simple proportions of 12-month and 24-month SR for evaluable patients were 70.8% (46 of 65) and 76.9% (30 of 39), respectively.

The majority of stricture recurrences (70.3%) developed within 8 months of substitution urethroplasty, and diminished gradually thereafter (Fig. 2). In the case of recurrence, it was not stated, if the new stricture was in the graft itself, or only at the proximal or distal anastomotic end.

After catheter removal, 92.6% (75 of 81 evaluable patients) were able to spontaneously micturate compared with 70.8% (52 of 72) at baseline. Preoperatively, mean ± SD Qmax was 8.3 ± 4.7 mL/s (n = 57) increasing to 25.4 ± 14.7 mL/s (n = 51) following catheter removal.

Presence of concomitant diseases (cardiovascular, pulmonary, oncological, inflammatory, orthopedic, diabetes mellitus), and smoking habits (never, stopped, active) did not significantly affect outcome as confirmed by Kaplan-Meier analyses (data not shown).

By Kaplan-Meier analysis, the comparison of RFS at 12 months in strictures with localization in the bulbar versus penile localization showed only tendencies in favor of bulbar strictures (p = 0.200). Univariate regression identified duration of catheterization as associated

Table 2
Overall success rate[a] and by study center at 12 months (Kaplan-Meier estimates).

Center	N	Success	95% CI
Overall	98[b]	67.3%	57.6–77.0
1	8	85.7%	59.7–100
2	10	80.0%	55.2–100
3	27	72.3%	54.7–89.9
4	23	69.3%	50.3–88.3
5	6	66.7%	28.9–100
6	13	56.4%	27.2–85.6
7	6	50.0%	10.0–90.0
8	5	0%[c]	–

CI = confidence interval.
[a] Proportion of patients in full analysis set without stricture recurrence.
[b] One patient with first assessment after 12 months was excluded.
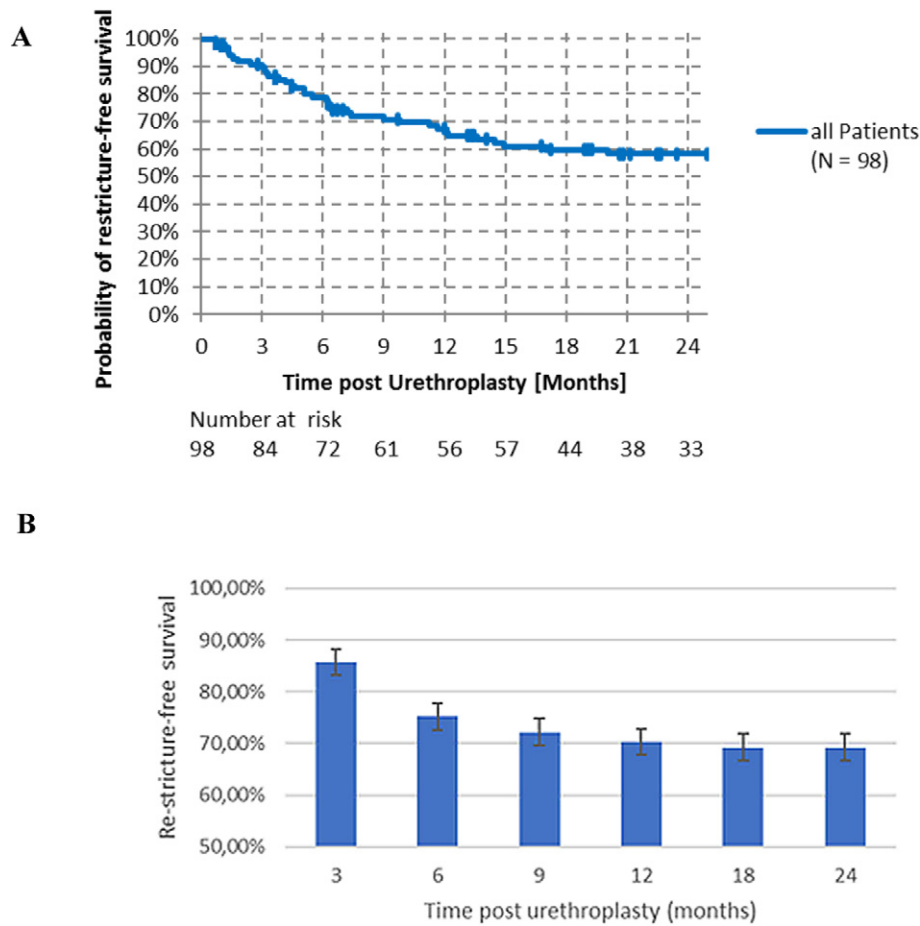[c] 3 of 5 patients failed, 2 were censored.

Fig. 2. Kaplan-Meier plot of re-stricture-free survival. Time calculated from date of urethroplasty surgery. One patient with first assessment after 12 months was excluded from analysis. Urethral strictures of any etiology, location, length and severity were included in the study. Re-stricture-free survival rate, based on uncensored data, using age-related Qmax (Ortega & Pena, 2009) as measure for stricture recurrence.

with stricture recurrence at 12 months and the number of prior surgeries as risk factor for re-stricture at 24 months, showing a hazard ratio (HR) (95% CI) of 2.23 (1.20–4.10; p = 0.010) and 1.74 (1.02–2.93; p = 0.039), respectively (Table 3). On multivariate analysis, timing of catheter removal and number of prior stricture surgeries remained statistically significantly associated with treatment failure, indicating that both are independent risk factors for the development of stricture recurrence.

Fig. 3 shows the Kaplan-Meier plot of re-stricture-free survival by number of prior surgeries. Long-term SR were best in patients with 1 previous surgical treatment and worst in patients with a history of ≥4 surgical treatments (urethrotomy or urethroplasty) (p = 0.019 for 1 vs. ≥4 prior treatments, log-rank test). Only one patient had no prior treatment rendering it impossible to do comparisons against patients without surgical pre-treatments. Kaplan-Meier analysis of re-stricture-free survival by duration of catheterization showed that patients with

catheter removal ≥ 28 days post-surgery exhibited the highest risk of treatment failure (p = 0.018 for ≤20 vs. ≥28 days, log-rank test).

Stricture length, age, and body mass index were not significantly associated with stricture recurrence.

No adverse event occurred at the oral mucosa harvesting site. Upon inquiry, three patients reported transient mild pain at the donor site. One patient was reported to have an unspecified complaint.

Treatment-related adverse events and serious adverse events other than stricture recurrence, which were reported by the investigators, are presented in Table 4.

Ninety patients were assessed for urethral pain within 10 days of urethroplasty surgery. Upon inquiry, 57 (63.3%) reported no pain, 33 (36.7%) had mild pain, and 4 (4.4%) experienced moderate pain. Immediately after catheter removal, 10% of patients reported mild urethral pain which subsided in most patients by month 3. At and beyond 3 months after urethroplasty, transient mild urethral pain was reported

Table 3
Univariate analysis of risk factors for stricture recurrence (based on uncensored data).

Factor	N	12 months HR (95% CI)	p value	24 months HR (95% CI)	p value
Age	96	0.85 (0.52–1.36)	0.492	0.98 (0.63–1.51)	0.918
Number of prior surgeries[a]	82	1.42 (0.81–2.48)	0.211	1.74 (1.02–2.93)	0.039
BMI	94	1.39 (0.81–2.40)	0.231	1.31 (0.80–2.14)	0.282
Stricture length	90	1.02 (0.63–1.64)	0.942	1.19 (0.76–1.85)	0.438
Duration of catheterisation	93	2.23 (1.20–4.10)	0.010	1.47 (0.88–2.50)	0.135

BMI = body mass index; CI = confidence interval; HR = hazard ratio.
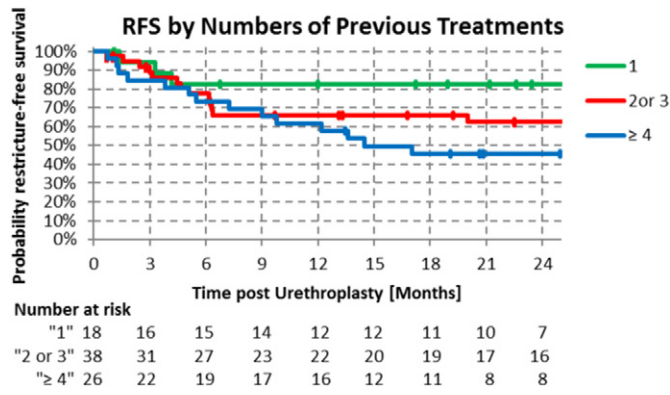[a] Urethrotomy and/or urethroplasty.

Fig. 3. Kaplan-Meier plot of re-stricture-free survival by number of previous surgeries (urethrotomy or urethroplasty). One patient with first assessment after 12 months was excluded from analysis. Urethral strictures of any etiology, location, length and severity were included in the study.

sporadically only (1–3 patients). Vital signs measurements and electrocardiogram assessments did not reveal clinically relevant findings. Physical examination findings were consistent with the underlying disease and the applied surgical procedure, or were considered unrelated to TEOMG implantation.

6. Discussion

We have shown that use of a TEOMG for reconstruction of the bulbar and penile urethra is feasible, safe, and efficacious in a heavily pre-treated population. In our multicenter, prospective, observational study in a non-preselected cohort, we observed a satisfactory clinical outcome after 12 and 24 months in the majority of patients.

Prospective multicentral studies of urethroplasty are rare (Mundy, 2006; Tritschler et al., 2013). A recent meta-analysis of 10 cohort studies of buccal mucosa graft urethroplasty and end-to-end anastomosis in short segment bulbar urethral stricture reported a recurrence rate of 30% (Yuri et al., 2016). The current survey, evaluated by an independent CRO, included complex penile and longer strictures (Liu et al., 2016; Ortega & Pena, 2009; Bello, 2016; Breyer et al., 2010; Yalcinkaya et al., 2015), and excluded end-to end anastomosis (known to have a very high SR) (Ortega & Pena, 2009; Ivaz et al., 2017). One can therefore consider the results of our study in general accordance with buccal mucosa urethroplasty (Yuri et al., 2016; Chauhan et al., 2016).

Previous studies in urethroplasty usually were retrospective, conducted at specialized single institutions, and operations were done by well-experienced surgeons (Liu et al., 2016; Chauhan et al., 2016; Barbagli et al., 2005; Kulkarni et al., 2012; Andrich & Mundy, 2008). Consequently, the reported SR was expectably high. In our study, clinics with varying levels of experience in urethral reconstruction surgery were included, displaying the heterogenic national urethral stricture population and routine practice patterns. Therefore, our data may describe more closely the use of urethroplasty and its effectiveness

Table 4
Reported events, considered as adverse events and serious adverse events by the investigators.

Adverse event	n	Time point
Local dermal infection	2	Postoperatively (Liu et al., 2016)

Serious adverse event	n	Time point
Urinary tract infection	2	6 weeks and 16 months
Ureter stone	1	2 months
Crohn's disease	1	2 months
Pulmonary embolism	1	Postoperatively
Epileptic seizure	1	Postoperatively
Death	1	34 months

under real world conditions. Data were collected prospectively, by using a standardized case report form, and, if necessary, followed-up by phone. They were monitored and evaluated by an independent clinical research organization and therefore represent a higher level of evidence (Latini et al., 2014; Bellomo & Bagshaw, 2006) (level 2, as adapted by the International Consultation on Urological Disease from the Oxford Centre for Evidence-Based Medicine) (Latini et al., 2014), compared to preceding studies not exceeding level 3 (Wessells et al., 2017; Latini et al., 2014; Mundy, 2006; Tritschler et al., 2013), or recommendations mostly based on expert opinion (evidence level 4) (Latini et al., 2014). From these, a MukoCell® drug registry was established, comprising real-world urethroplasty results.

The patient population in our study mostly had undergone multiple previous urethral surgeries (urethrotomy and/or urethroplasty) which failed. A high number of dilatations were also reported (127 preoperative dilatations in 10 patients, multiple dilatations in 8, no dilatations in 6 and unknown in the remaining patients) but not assessed in our statistical evaluations. Due to the high number of prior failed treatments in the study cohort and the known correlation with stricture recurrences (Liu et al., 2016; Bello, 2016; Breyer et al., 2010; Kulkarni et al., 2012), the presented overall success rate is, as can be expected for urethroplasty with native oral mucosa. The subgroup analysis regarding number of prior surgeries (Fig. 3) confirms this, displaying a much higher success rate of 83.3% in the patients with one prior surgery.

There were notable differences in SR between centers with the general tendency that sites recruiting more patients had a higher SR. This may be attributable to the learning curve in performing TEOMG implantation as reported for other urological procedures (Abboudi et al., 2014). Heterogenous levels of urethroplasty experience (SR ranging between 0% to 85.7%) as well as varying post-surgical management could also have an influence. Additionally, some of the investigators have included patients with less complex urethral stricture diseases (Alwaal et al., 2016) while other surgeons used TEOMG implantation as the very last therapeutic option, which was associated with an increased risk of stricture recurrence. Unclear filling of some report forms by urologists have resulted in the statistical rating of some successful outcomes as recurrences. Heterotopic strictures, postoperative urinary flows of 16 ml/s and 27 ml/s, and missing case report forms, in a total of six patients, have been assessed as stricture recurrences in the statistical analysis, due to the conservative GCP approach. These exemplarily stand for the difficulties and limitations of applying GCP principles to an observational trial, with its intrinsic grades of freedom. Finally, during the post-operative follow-up time, 1 urinary tract infection (Navai et al., 2008) with febrile temperature, may have caused a recurrence.

Among urologists, some controversy exists about the time for catheter removal following urethroplasty (Al-Qudah et al., 2005). Based on the presented results, we suggest that after TEOMG implantation, the indwelling catheter should not be left in place for longer than 3–4 weeks.

We could not identify stricture length as independent risk factor for treatment failure, which may indicate that a TEOMG implant tailored according to individual needs favors successful outcome largely independent from stricture length.

We did not detect unexpected adverse events related to TEOMG implantation, and particularly, the reported serious adverse events were evidently unrelated to the TEOMG itself, but rather to the implantation surgery in general. Only one adverse event (mild, transient pain) occurred at the donor site of oral mucosa, indicating the virtual absence of donor-site morbidity.

The excision of large segments of native oral mucosa results in long-term (>12 months) side-effects in about 20% of the cases, e.g. scars and oral contractures (Jang et al., 2005; Fasolis et al., 2014; Markiewicz et al., 2008). Chronic mechanical irritations from ill-fitting dentures and dental rubbings (Perry et al., 2015) as well as parafunctional bitings of oral mucosa (Piemonte et al., 2010) bulges have a high association in the development of oral cancer. The use of TEOMG would provide the

possibility of avoiding this (Perry et al., 2015; Piemonte et al., 2010) and other (Jang et al., 2005; Fasolis et al., 2014; Markiewicz et al., 2008) potential risks associated with the native oral mucosa excision. Additionally, the use of the TEOMG is associated with reduction of anesthesia and operation time. This is of special meaning regarding elderly patients, who are on particular risk regarding anesthesia and wound healing impairment. Due to the fact, that it was possible to produce even for patients above 80 years a high quality TEOMG without any signs of reduced cell proliferation and viability, urethroplasty with TEOMG may also be considered in these patients.

Our study has several limitations. Missing randomization and control group may have caused bias. However, the results were collected prospectively, and independent source data monitoring and verification took place. There was no standard objective measure for stricture recurrence. However, the use of a consolidated criterion for treatment failure considering all available information may put this limitation into perspective. Urethroplasties were performed by different surgeons with heterogenous levels of experience, which may represent a confounding factor. Finally, 98 of 99 enrolled patients were surgically pre-treated rendering it impossible to perform a historical comparison of stricture-free survival against patients who underwent first substitution urethroplasty.

Another limitation of the study was that the degree of spongiosal fibrosis and the presence of obliterative strictures preoperatively, known to be correlated with success and failure, were not recorded in most cases. Similarly, the sites of the new strictures (if full length of the graft, only anastomosis site, or if heterotopic) were not clearly documented in the majority of cases and have been evaluated as therapy failures.

The stability of the graft quality is an important aspect of all ATMPs. For the authorization of a tissue-engineered product, stability data must be provided to the authorities, showing that the quality of the product remains unchanged during shipment. This is shown for the TEOMG over a period of 48 h. The fact that The TEOMG must be applied within this time window, may be considered as a limitation of the product.

In tissue engineering, different grafts with one or more cell types have been investigated. Previous scientists (Butler & Orgill, 2005) have shown that autologous epithelial cells, cultured on a matrix, regenerate not only the epidermis, but also the dermis after implantation. Additionally, they showed that during regeneration, a basement membrane of normal appearance forms at the dermo-epidermal junction. Regarding urethral regeneration, we have shown in a previous study (Ram-Liebig et al., 2015) in pigs the complete regeneration of the urethral tissue a few weeks after the implantation of TEOMG. The epithelialization of the wound bed by implanted epithelial cells, which are known to produce different cytokines, seems to stimulate mechanisms to initiate the process of wound healing and tissue regeneration in the subepithelial layers.

For the manufacture of TEOMG, precise coordination between the manufacturing site and the clinic is mandatory. One of the challenges in this procedure is the Good Professional Practice conform documentation of biopsy taking by the physician. After getting a respective training and performing the documentation a few times, the procedure usually becomes routinized. Another challenge is that exact coordination between the urologist, the patient and the GMP laboratory is required. The duration of the manufacturing process for some ATMPs is not predictable and operations can only be scheduled on short terms. Nevertheless, in the case of TEOMG, a robust and reproducible process was developed, which resulted in an absolute strict time interval for all batches produced so far.

In the manufacture of ATMPs, difficulties in biopsy taking and graft preparations may occur, resulting in changings in patient's outcome and statistical difference in terms of change in success rate. However, due to the standardized manufacture and consistent quality of TEOMG, these difficulties did not occur. The consistent quality of TEOMG may even be considered as a superiority of this graft versus native oral mucosa, which may rather have varying quality.

For ATMPs, as for all therapeutic measures, the benefit-cost relation must be positive and must be assessed for each product individually. In the case of the TEOMG the benefit consists in the avoidance of a second operation, namely the excision and potentially damage of healthy oral mucosa. Today, tissue engineering technology helps to avoid the additional oral intervention. Nevertheless, ATMPs cannot be delivered for free. In fact, unlike for native oral mucosa, strict rules for quality and safety standards must be kept for ATMPs like TEOMG. Moreover, to gain market access, a high level of clinical evidence is required, and therefore cost-intensive and time-consuming clinical studies must be carried out. We are now faced with the decision between a graft, which indeed is cost-free but requires the excision and more or less the damage of healthy pieces of the body, partly with severe complications, and a product, which is not cost-free, but helps to avoid the additional intervention. From an ethical point of view, physical integrity is a precious good and has a high value. To our opinion we should try, not to sacrifice the integrity of the body just because of cost saving. The best solution in the case of TEOMG would therefore be the short-term reimbursement of this product by health insurances.

In conclusion, we have shown that TEOMG represents a safe and efficient alternative to native oral mucosa as a graft for surgical substitution of narrowed urethra, which may spare the patients risk and discomfort at the intra-oral donor site. Furthermore, our results suggest that surgical substitution should be performed early in disease before interventions repeatedly have failed, and that the surgeon's experience and appropriate post-surgical management (e.g. early catheter removal) are key for a favorable outcome.

Conflict of Interests

GRL is an employee of UroTiss Europe GmbH. Guido Barbagli is advisor for UroTiss Europe. The other authors declare no competing interests.

Registration

Paul-Ehrlich-Institut observational trial registry, NIS number 110.

Author Contributions

GRL did the literature search and wrote the report. The remaining authors performed surgical operations and were involved in patient recruitment, follow-up, data collection and patient care. They also contributed to the design of the study and assisted with data interpretation. GB and GR operated at German centers. All authors revised the report and approved the final version.

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